PATENT

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In re Application of: Barney GRAHAM, Tara GOWER, and Manoj PASTEY	Group Art Unit: 1617
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CERTIFICATE OF ELECTRONIC SUBMISSION
DATE OF SUBMISSION: January 18, 2007

COMMUNICATION

Commissioner for Patents
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Sir:

This Communication is filed to submit the attached paper by Grower & Graham (2001) which was inadvertently left from the submission of the Amendment and Request for Reconsideration Under 37 C.F.R. § 1.111 via electronic submission on December 21, 2006. Should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from Fulbright & Jaworski L.L.P. Account No.: 50-1212/VBLT:003US/SLH.

Should the examiner have any questions regarding this response, a telephone call to the undersigned is invited.

Respectfully submitted,

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Antiviral Activity of Lovastatin against Respiratory Syncytial Virus In Vivo and In Vitro

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Respiratory syncytial virus (RSV) is an important human pathogen that can cause severe and life-threatening respiratory infections in infants and immunocompromised adults. We have recently shown that the RSV F glycoprotein, which mediates viral fusion, binds to RhoA. One of the steps in RhoA activation involves isoprenylation at the carboxy terminus of the protein by geranylgeranyltransferase. This modification allows RhoA to be attached to phosphatidyl serine on the inner leaflet of the plasma membrane. Treatment of mice with lovastatin, a drug that inhibits prenylation pathways in the cell by directly inhibiting hydroxymethylglutaryl coenzyme A reductase, diminishes RSV but not vaccinia virus replication when administered up to 24 h after RSV infection and decreases virus-induced weight loss and illness in mice. The inhibition of replication is not likely due to the inhibition of cholesterol biosynthesis, since gemfibrozil, another cholesterol-lowering agent, did not affect virus replication and serum cholesterol levels were not significantly lowered by lovastatin within the time frame of the experiment. Lovastatin also reduces cell-to-cell fusion in cell culture and eliminates RSV replication in HEp-2 cells. These data indicate that lovastatin, more specific isoprenylation inhibitors, or other pharmacological approaches for preventing RhoA membrane localization should be considered for evaluation as a preventive antiviral therapy for selected groups of patients at high risk for severe RSV disease, such as the institutionalized elderly and bone marrow or lung transplant recipients.

Human respiratory syncytial virus (RSV) belongs to the family *Paramyxoviridae* and is the leading viral cause of severe lower respiratory tract illness in infants and young children (37). RSV can also cause severe illness and death in the elderly (35) and immunocompromised bone marrow (12, 38) and lung transplant (38) patients. The mortality rate for bone marrow transplant patients is between 70 and 100% (12). Although RSV-induced disease in infants may be primarily immune mediated, in bone marrow and lung transplant recipients and in persons with severe combined immunodeficiency syndrome the pathology, characterized by giant cell formation, is related to ongoing viral replication. In addition, infants with AIDS have been shown to have continuous viral shedding for more than 200 days (15). These patient groups would benefit from more effective antiviral therapeutic options for RSV. It is more likely that antiviral prophylaxis would be required to make an impact on illness in infants and the elderly.

We have previously demonstrated that the fusion (F) glycoprotein from RSV interacts with RhoA, a small GTP binding protein in the Ras superfamily, which is ubiquitously expressed in mammalian cells (26). F is required for cell-to-cell fusion and syncytium formation and is thought to be required for virus entry into cells, but the exact mechanisms of virus-induced membrane fusion have not been defined (22). A peptide containing amino acids 77 to 95 of this region was highly efficient in blocking infection and syncytium formation in vitro and in vivo (27).

RhoA influences a variety of essential biological functions in eukaryotic cells, including gene transcription, cell cycle, vesicular transport, adhesion, cell shape, fusion, and motility, through its activation of signaling cascades (34). RhoA has also been shown to regulate smooth muscle contraction via Rho kinase (p160 ROCK), causing airway hyperresponsiveness. This is of particular interest because of the association of RSV with childhood asthma (32, 33). Cytoplasmic RhoA is activated by an exchange of GTP for GDP and by attachment to the intracellular side of the plasma membrane after isoprenylation by geranylgeranyltransferase at the carboxy-terminal cysteine of the protein (1, 6, 13, 19, 23). Activation of RhoA in a cell affects production of several cytokines, such as interleukin-1-beta (IL-1β), IL-6, and IL-8, which are produced by RSV-infected cells (4), and alters cytoskeletal structure by inducing organization of actin stress fibers and formation of focal adhesion plaques (11, 20, 28, 34). We have shown that RhoA is activated by RSV infection and that inactivating RhoA with C3 toxin from *Clostridium botulinum*, which ADP ribosylates RhoA on Asn 41, prevents RSV-induced syncytium formation (T. L. Gower et al., unpublished observation; 29, 31). Therefore, we postulate that RhoA-mediated signaling may play a role in various aspects of RSV pathogenesis, including cell-to-cell fusion, secretion of IL-1β, IL-6, and IL-8, and airway hyperresponsiveness.

Lovastatin is an FDA-approved drug that is used to treat hypercholesterolemia. It inhibits hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, an important enzyme in the cholesterol biosynthesis pathway (2, 3, 7, 18, 25). Lovastatin is also used to study isoprenylation and membrane localization of proteins such as RhoA, since a branch of the cholesterol biosynthesis pathway leads to the formation of isoprenyl groups (16, 24). Lovastatin inhibits the production of geranyl geranyl,

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pyrophosphate and farnesyl pyrophosphate and therefore inhibits protein isoprenylation (2, 3).

We asked whether lovastatin could affect RSV replication in mice and in cell culture. We show that RSV replication is attenuated by lovastatin in mice and in cell culture assays. Lovastatin can also decrease virus-induced illness in mice. Based on findings in this paper, agents that prevent isoprenylation may represent a new class of antiviral drugs that should be considered for clinical evaluation in selected groups.

MATERIALS AND METHODS

Virus and cells. The A2 strain of RSV was provided by R. Chanock, National Institutes of Health, Bethesda, Md. RSV stocks were prepared as previously described (10). HEp-2 cells were maintained in Eagle's minimal essential medium supplemented with glutamine, gentamicin, penicillin G, and 10% fetal bovine serum.

Plaque assay. Two-day-old HEp-2 monolayers, 80% confluent in 12-well plates (Costar, Cambridge, Mass.), were used for the RSV plaque assay. The assay was done as previously described (10).

Mouse studies. A dose of 1 mg of lovastatin/day was chosen for the mouse experiments after a dose-response experiment. The dose for gemfibrozil (50 mg/day) was chosen because it is a dose equivalent to 1 mg of lovastatin/day in humans. Twelve-week-old female pathogen-free C57BL/6 or BALB/c mice (Harlan-Sprague Dawley, Indianapolis, Ind.) were given 1 mg of lovastatin (Merck, Rahway, N.J.)/day in 100 μl of phosphate-buffered saline (PBS), 50 mg of gemfibrozil (UDL, Rockford, Ill.)/day in 200 μl of PBS, or 100 μl of PBS only by oral gavage starting at various times prior to and after virus infection and throughout the course of the experiment. Mice were anesthetized and infected intranasally with 10^7 PFU of RSV or 10^5 PFU of vaccinia virus. Lungs were harvested for RSV and vaccinia plaque assays, as previously described, 4 days after RSV infection (10).

Cell fusion assay using vaccinia virus-based expression of RSV envelope glycoproteins. The ability of lovastatin to inhibit RSV-induced cell-to-cell fusion was assessed using a fusion assay. One population of HEp-2 cells was infected with recombinant vaccinia virus vTF7-3, which encodes T7 polymerase, at a multiplicity of infection of 10 PFU per cell and was transfected with plasmids encoding RSV glycoproteins F, G, and SH under control of the T7 promoter (gifts from P. Collins, National Institutes of Health) using FuGene (Boehringer Mannheim, Indianapolis, Ind.). At 5 h after transfection, the cells expressing viral envelope proteins were trypsinized, suspended in minimal essential medium containing 2.5% fetal bovine serum to a density of 2×10^5 cells per ml, and incubated overnight at 32°C. The cells were then washed and suspended in Opti-MEM (Gibco BRL, Grand Island, N.Y.) at a concentration of 10^6 cells per ml. A second population of HEp-2 cells was infected with recombinant vaccinia virus expressing β-galactosidase under control of the T7 promoter (provided by E. A. Berger, National Institutes of Health). The cell population infected with recombinant vaccinia virus expressing β-galactosidase was split in half. Half of the cells were left untreated, and the other half were treated with 20 μM lovastatin for 24 h beginning at the time of infection. At 5 h after infection, cells were trypsinized and finally suspended at a concentration of 10^5 cells per ml. The two cell populations were mixed in triplicate by adding 100 μl of each cell population to 96-well tissue culture plates, which were then incubated at 37°C for 4 h. At 4 h the cells were fixed in 2% glutaraldehyde–20% formaldehyde (Sigma, St. Louis, Mo.) in PBS for 10 min. One hundred fifty microliters of X-Gal solution (1 M potassium ferricyanide, 1 M potassium ferrocyanide, 1 M magnesium chloride, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside [X-Gal] [Fisher, Springfield, N.J.]) was added. After 8 h, blue-stained fused cells were viewed with an inverted phase-contrast microscope.

Statistical analysis. Data from individual experiments were maintained in a Paradox database. Statistical analysis was performed by transferring data from the database into the SAS (Chapel Hill, N.C.) statistical software to perform analysis of variance using Kruskal-Wallis and Wilcoxon rank sum tests. Comparisons were made between individual experiments by using statistical modeling and trend analysis calculated by the general linear model method in the SAS package. Comparison of means between two groups was then performed using the Student's *t* test. *P* values of less than 0.05 were considered statistically significant.

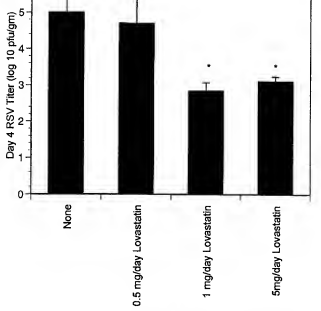

FIG. 1. RSV replication in lovastatin-treated mice. C57BL/6 mice were give 0.5, 1, or 5 mg of lovastatin/day by oral gavage beginning 3 days prior to RSV infection and for 8 days after infection. Mice were infected intranasally with RSV at day 0, and lungs were harvested at day 4 for plaque assay. Each group represents five mice, and error bars represent standard deviations. The data were subjected to statistical analysis, and asterisks represent statistically significant changes.

RESULTS

Lovastatin diminishes RSV replication in mice. To determine if lovastatin could inhibit RSV replication in vivo, C57BL/6 mice were subjected to a dose-response curve from 0.5 to 5 mg of lovastatin/day to determine the optimal concentration for inhibition of RSV (Fig. 1). Mice treated with 1 mg of lovastatin/day and infected with RSV had a peak titer in the lung of 2.9 ± 0.26 ($\log_{10}$ PFU/g), and RSV-infected mice treated with 5 mg of lovastatin/day had a peak titer in the lung of 3.1 ± 0.14 ($\log_{10}$ PFU/g), compared to lovastatin-treated (0.5 mg/day) and untreated RSV-infected mice, which had peak viral titers of 4.7 ± 1.06 and 5.0 ± 0.74 ($\log_{10}$ PFU/g), respectively (Fig. 1). The mice treated with 1 mg of lovastatin/day and 5 mg of lovastatin/day had significantly lower viral titers than untreated mice, with *P* values of 0.001 and 0.002, respectively. Since doses of 1 and 5 mg/day inhibited RSV replication significantly and similarly, we chose to continue the studies using 1 mg of lovastatin/day. To determine the specificity of lovastatin for RSV, mice were treated with 1 mg of lovastatin/day, 50 mg of gemfibrozil/day, or PBS by oral gavage beginning 3 days prior to infection with either RSV or vaccinia virus. Vaccinia replication (Fig. 2) and illness (data not shown) were not affected by lovastatin or gemfibrozil treatment compared to results for PBS-treated controls. Gemfibrozil- and PBS-treated mice infected with RSV had peak titers in the lung of 6.5 ± 0.43 ($\log_{10}$ PFU/g) and 6.5 ± 0.19 ($\log_{10}$ PFU/g), respectively, while RSV replication in lovastatin-treated mice

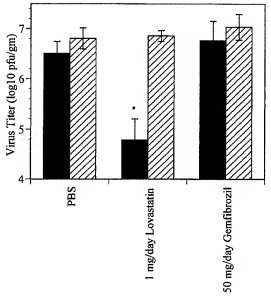

FIG. 2. Lovastatin diminishes RSV replication in mice. BALB/c mice were given 1 mg of lovastatin/day, 50 mg of gemfibrozil/day, or PBS by oral gavage starting 3 days prior to RSV or vaccinia virus infection and for 8 days after infection. Mice were infected intranasally with RSV (solid bars) or vaccinia virus (hatched bars) at day 0. Lungs were harvested at day 4 for RSV and vaccinia plaque assays. Each group represents five mice, and error bars represent standard deviations. The data were subjected to statistical analysis, and the asterisk represents a statistically significant change.

was reduced by nearly 100-fold, to 4.7 ± 0.4 ($\log_{10}$ PFU/g), compared to results for untreated RSV-infected mice (Fig. 2). Statistical analysis showed that lovastatin significantly reduced viral titers of RSV compared to results for PBS-treated mice, with a P value of <0.0001.

To determine if lovastatin could effectively inhibit virus replication if given after infection, mice were treated with 1 mg of lovastatin/day beginning either 3 days prior to infection, 1 day prior to infection, 1 day after infection, or 3 days postinfection (Fig. 3). Untreated mice and mice given lovastatin starting 3 days after RSV infection had similar viral titers in the lung on day 4 of 6.2 ± 0.79 ($\log_{10}$ PFU/g) and 6.3 ± 1.1 ($\log_{10}$ PFU/g), respectively (Fig. 3). Mice treated with lovastatin beginning 3 days prior to infection had a more than 100-fold reduction in the viral titer, to 3.8 ± 0.48 ($\log_{10}$ PFU/g), compared to results for untreated mice, with a P value of 0.0008 (Fig. 3). Lovastatin was also able to reduce RSV replication when mice were treated soon after infection. For mice treated beginning 1 day after RSV infection, viral titers were reduced by 10-fold, which is a significant reduction compared to results for untreated mice, with a P value of 0.04. However, mice treated with lovastatin beginning 3 days after infection showed no reduction in viral titers compared to results for untreated mice.

Lovastatin diminishes RSV-induced illness in mice. Mice were also weighed daily and given illness scores as a measure of illness. Mice treated with lovastatin 3 days after RSV infection had a similar weight loss curve (Fig. 4) and similar illness scores (data not shown) compared to untreated RSV-infected mice. Both groups had a peak weight loss of about 30% at 8 days postinfection (Fig. 4). Mice treated with lovastatin at earlier time points in the infection showed a reduced level of illness. Weight losses at 8 days postinfection for mice treated with lovastatin 3 days prior to infection, 1 day prior to infection, and 1 day postinfection were 17, 19, and 22%, respectively (Fig. 4). These reductions in RSV-induced illness are statistically significant compared to results for untreated mice 8 days postinfection, with P values of 0.014, 0.04, and 0.05, respectively. Therefore, the degree of inhibition by lovastatin was dependent on the time treatment was started. RSV-induced illness was also diminished in lovastatin-treated mice compared to results for untreated mice or gemfibrozil-treated mice, as measured by the percent weight loss. Mice treated with PBS, gemfibrozil, and lovastatin had peak weight losses on day 8 postinfection of 27, 40, and 19%, respectively (data not shown). Uninfected mice treated with either 1 mg of lovastatin/day or 50 mg of gemfibrozil/day for 11 days did not show a significant change in weight during the experiment (Fig. 4). Therefore, the drug treatments alone are not toxic to the mice.

Lovastatin does not affect serum cholesterol levels during acute infection. Since lovastatin reduces total levels of cholesterol in serum over time, we wanted to determine if this could be the cause of reduced RSV replication in mice. To determine whether lovastatin could reduce serum cholesterol levels in the time frame of this experiment, serum samples were collected from mice treated 3 days before infection, 1 day before infection, 1 day after infection, and 3 days after infection and from untreated mice 8 days post-RSV infection. Serum cholesterol levels were measured using the ACE7 Cholesterol Reagent. There were no significant differences in serum cholesterol levels between groups (data not shown).

Lovastatin eliminates RSV replication in HEp-2 cells. Next, we asked whether lovastatin could alter RSV replication in cell

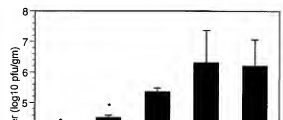

FIG. 3. Inhibition by lovastatin is dependent on the duration of the treatment. BALB/c mice were given 1 mg of lovastatin/day by oral gavage at several time points throughout the course of RSV infection, beginning either 3 days prior to RSV infection, 1 day prior to infection, 1 day postinfection, or 3 days postinfection. Control mice were infected with RSV but not treated with lovastatin. Lungs were harvested 4 days after RSV infection for viral plaque assay. Each group represents five mice, and error bars represent standard deviations. The data were subjected to statistical analysis, and asterisks represent statistically significant changes.

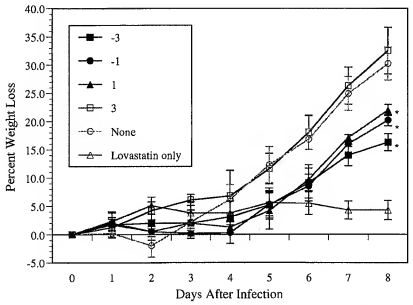

FIG. 4. Lovastatin diminishes RSV-induced weight loss in mice. BALB/c mice were given 1 mg of lovastatin/day beginning either 3 days prior to RSV infection (−3), 1 day prior to infection (−1), 1 day postinfection (1), or 3 days postinfection (3). Untreated mice were also infected with RSV. Mice were weighed daily, and the percent weight loss was calculated. Each group represents three mice, and error bars represent standard deviations. The data were subjected to statistical analysis, and asterisks represent statistically significant changes.

culture. HEp-2 cells in a 96-well plate were either treated with 10 μM lovastatin or left untreated beginning 24 h prior to RSV infection. The contents of an individual well were transferred to HEp-2 monolayers in 12-well plates for plaque assay for eight consecutive days after RSV infection (Fig. 5). RSV replicates normally in untreated HEp-2 cells, with viral replication peaking on days 4 to 6 post-RSV infection. Interestingly, RSV replication is completely inhibited in lovastatin-treated cells. Replication is restored in cells that have been treated with 10 μM lovastatin for 24 h followed by treatment with 20 μM mevalonolactone, which rescues the cholesterol biosynthetic pathway just downstream of HMG-CoA reductase (data not shown). This indicates that the lovastatin effect on RSV replication is mediated by the products of this biosynthetic pathway and not by alternative mechanisms.

Since lovastatin has a broad effect on cells, we performed viability tests using trypan blue exclusion in cells treated with various concentrations of lovastatin for 24 h. We calculated the lethal dose for 50% of the cells to be 74.5 μM (data not shown). To calculate the concentration of lovastatin that inhibits 50% of RSV infection, we treated cells with several concentrations of lovastatin, from 50 μM to 0 μM, for 24 h prior to infection and counted the number of RSV-induced syncytia (data not shown). The 50% inhibitory concentration for lovastatin is 3.1 μM. In addition, cells could be treated with lovastatin for 24 h, washed, and then infected with RSV without plaque formation (data not shown).

We next tested the effect of lovastatin on influenza virus replication to determine whether lovastatin was specific for paramyxoviruses. MDCK cells were treated with 10 μM lovastatin beginning 24 h prior to infection with influenza virus, and plaque formation was not inhibited (data not shown).

Lovastatin diminishes cell-to-cell fusion. Next we asked whether lovastatin could inhibit RSV-mediated cell-to-cell fusion. We used a fusion assay in which one set of HEp-2 cells was transfected with the RSV envelope proteins F, G, and SH

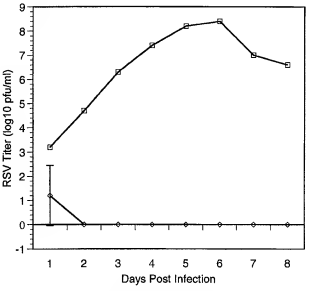

FIG. 5. Lovastatin eliminates RSV replication in cell culture. HEp-2 cell monolayers grown in 96-well plates were either treated with 10 μM lovastatin or left untreated beginning 24 h prior to RSV infection (multiplicity of infection, 0.1). Virus titers were measured daily for eight consecutive days after RSV infection by harvesting the entire contents of a well and performing plaque assays in triplicate. RSV growth curves for untreated cells (□) and cells treated with 10 μM lovastatin (◇) are shown. Error bars represent standard deviations.

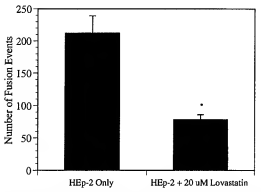

FIG. 6. Lovastatin diminishes cell-to-cell fusion. HEp-2 cells in 96-well plates were either treated with 20 μM lovastatin and infected with recombinant vaccinia virus expressing the β-galactosidase gene under the direction of T7 polymerase or left untreated at the time of infection. Cell-to-cell fusion was determined by calculating the average number of blue cells. Each error bar represents the standard deviation calculated from the average number of fusion events in eight separate wells. Lovastatin significantly reduces cell-to-cell fusion, with a P value of 0.004.

and infected with a recombinant vaccinia virus expressing T7 polymerase. Another set of HEp-2 cells was infected with a recombinant vaccinia virus encoding a *lacZ* gene under the direction of a T7 promoter. One-half of the cells containing the *lacZ* gene were treated with 20 μM lovastatin at the time of vaccinia infection. The other half were left untreated. After mixing the two cell populations for 4 h, cells were fixed and X-Gal was added. The number of blue cells among lovastatin-treated and untreated cells was counted. Untreated cells had an average of 215 ± 29 fusion events per well, and lovastatin-treated target cells had an average of 75 ± 11 fusion events per well (Fig. 6). Therefore, lovastatin can diminish virus-induced cell-to-cell fusion by more than 50% when cells are treated 16 h prior to mixing the two cell populations. This effect can also be seen with cells that have been treated with 10 μM lovastatin and rescued by the addition of 20 μM mevalonolactone (data not shown). This indicates that localization of RhoA in the plasma membrane may be important for RSV-mediated cell-to-cell fusion.

DISCUSSION

HMG-CoA reductase inhibitors, like lovastatin, are currently used for treating hypercholesterolemia in humans (2, 3, 25). We now report data that show that lovastatin can inhibit RSV replication in vivo and in vitro. RSV causes severe lower respiratory infections in children (37), the elderly (35), and recipients of bone marrow (13, 39) and lung (38) transplants. There are limited therapeutic options available for RSV disease, and the mortality rates for immunocompromised patients and the elderly remain high (12, 35, 39). Since lovastatin inhibits several pathways in the cell, such as those for the production of cholesterol and isoprenyl groups, there are several possible mechanisms by which lovastatin could inhibit RSV replication. Lovastatin may inhibit isoprenylation of RhoA, thereby preventing its localization in the plasma membrane. If RSV F binding to RhoA is involved in the process of membrane fusion or intracellular signaling events, this could limit virus entry and potentially inhibit replication. Alternatively, it may lower the cholesterol content in the cell membrane and alter lipid microdomains, potentially interfering with either virus entry and membranc fusion or assembly and budding.

We show that for mice treated with lovastatin beginning 3 days prior to infection, peak virus titers in lungs are reduced 100- to 1,000-fold 4 days after RSV infection (Fig. 1, 2, and 3). In addition, lovastatin treatment reduces RSV-induced illness (data not shown) and weight loss (Fig. 4). Lovastatin decreases RSV replication and disease most effectively when given prior to infection or at very early stages of infection (Fig. 3 and 4). Since lovastatin does not inhibit vaccinia virus replication and gemfibrozil does not inhibit RSV replication (Fig. 2), we conclude that lovastatin is having a specific effect on RSV which is independent of cholesterol production. This is supported by the finding that lovastatin did not inhibit plaque formation in MDCK cells infected with influenza virus. Mice that were treated with 1 mg of lovastatin/day for 11 days had no significant change in serum cholesterol levels (data not shown). However, we cannot exclude the possibility that lovastatin is having another effect on the virus-cell interaction that inhibits RSV replication or the spread of RSV.

We have previously shown that the RSV F glycoprotein interacts with cellular RhoA and that RhoA-derived peptides can neutralize RSV infectivity (26, 27). RhoA is an essential host cell protein with GTPase activity and is known to influence a variety of signaling pathways and basic cell functions (11, 20). One of the steps in RhoA activation is geranylgeranylation at its carboxy terminus, which allows RhoA to be attached to the plasma membrane (1, 6, 13, 19). We have shown that activated RhoA and subsequent signaling events are required for RSV syncytium formation and viral filament formation (Gower et al., unpublished observation). Lovastatin can inhibit the production of isoprenyl groups, thereby preventing the geranylgeranylation of RhoA and its attachment to the plasma membrane (16, 17). Since an activated, membrane-bound RhoA is required for RSV syncytium formation, we asked whether lovastatin could inhibit cell-to-cell fusion in a fusion assay. Cells transfected and expressing RSV F, G, and SH, the three viral envelope proteins expressed on the surfaces of infected cells, are able to fuse with HEp-2 cells. Cell-to-cell fusion is reduced by more than 50% for HEp-2 cells treated with lovastatin (Fig. 6).

Although lovastatin has many other effects on cells other than the inhibition of RhoA isoprenylation, our data support our hypothesis that RhoA is important in the biology of RSV replication. RSV causes a distinct disease syndrome and a unique pattern of immune responses in some individuals. It is intriguing to consider whether some of these responses may be caused in part by the consequences of RhoA activation and downstream signaling events. These functions of RhoA may not be essential to RSV replication but may lead to disease manifestations associated with RSV. RhoA activation can also lead to smooth muscle contraction through a Rho kinase pathway (8, 9, 14). Interestingly, a clinical hallmark of RSV infection is wheezing, and severe disease is associated with childhood asthma (32). It is possible that RhoA-mediated contraction of airway smooth muscle may contribute to RSV-induced wheezing, since RhoA activation has been linked to

airway hyperresponsiveness (5). In addition, RhoA activation leads to the transcription of genes for IL-1β, IL-6, and IL-8 (20), which are produced at high concentrations by RSV-infected cells (4). Therefore, inhibiting RhoA activation in vivo may not only lessen virus-induced syncytium formation but also impact illness by diminishing RhoA signaling activity.

A second mechanism by which lovastatin may inhibit RSV replication is by disrupting cholesterol-rich lipid rafts, which can interfere with virus entry and fusion. Lipid rafts are membrane microdomains that contain cholesterol. Several cellular proteins, such as RhoA and CD44, localize predominantly to raft domains. Lovastatin can disrupt lipid rafts because it inhibits cholesterol synthesis. Lipid rafts have been shown to play an important role in entry and fusion of both human immunodeficiency virus type 1 (21) and influenza virus (30, 40). It is not known if RSV utilizes lipid rafts during replication, but RSV fuses directly to the plasma membrane by a pH-independent process with protein machinery similar to that used by human immunodeficiency virus type 1.

Our findings indicate that lovastatin can inhibit RSV replication and virus-induced cell-to-cell fusion. While the doses of lovastatin used for mice and cell culture may not be achievable with standard dose regimens in humans, these experiments demonstrate a proof-of-principle and suggest that isoprenylation inhibition is a novel antiviral approach that may be potentially useful in the treatment or prevention of severe RSV infection. We will focus our future efforts on defining the precise mechanisms by which lovastatin inhibits RSV replication and on evaluating more specific inhibitors of gcranylgeranylation (36) that are in development.

ACKNOWLEDGMENTS

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